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ORIGINAL ARTICLE

Relationship between relative skeletal muscle index (RSMI) in physically active adults and the use of major sports supplements: a retrospective observational study

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Abstract

Introduction: The indiscriminate use of supplements poses a problem for overall health, with Brazil being one of the countries that consumes the most dietary supplements in the world. However, when used as indicated and correctly, supplements can positively influence muscle mass gain, performance, and general health. **Objective:** This study aimed to evaluate whether there is a relationship between physical exercise and the use of supplements related to physical exercise that are most common in clinical practice (whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine) with relative skeletal muscle indices (RSMI). **Methods:** The present study employed a retrospective observational design, adhering to the STROBE guidelines. A total of 50 adult patients aged between 18 and 65 years who practice physical exercise were evaluated in medical centers in the city of Brasília between May 2023 and May 2024 using whole-body Bioimpedance Imaging or DEXA analysis. Only patients who used one or more supplements and were non-sedentary according to the criteria of the health organization were included in the study. **Results:** The general clinical data presented a total of 50 participants, the majority of whom were male (58%), with the DEXA group having 44 participants and the BIO group having 06 participants. The overall mean age was 40.60 years (22 to 60). The amount of physical exercises practiced per week was 4.80 (3 to 9) in general. It was also found that the overall RSMI had a mean of 8.56 ±1.76. The general use of supplements (DEXA and BIO) showed that the use of creatine (34%) and creatine with Whey Protein (24%) were the most frequent. The present study observed that there was an important relationship between the male gender and the RSMI, with OR=4.89 and p=0.000. In addition, the nominal logistic regression analysis of the supplement predictors in general (DEXA and BIO) to the RSMI response predictor showed statistical significance to the combinations of supplements creatine, BCAA, Whey, and Whey, creatine, and beta-alanine, p=0.015 and OR=1.62 for both combinations. There was no statistically significant difference between the mean RSMI values of the DEXA and BIO groups, with p=0.307. **Conclusion:** It was concluded that there was a relationship between physical exercise and the use of supplements most common in clinical practice, such as Whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine with the increase in the relative skeletal muscle mass index (RSMI) in physically active adults. The general use of supplements (DEXA and BIO) showed that the use of creatine (34%) and creatine with Whey Protein (24%) were the most frequent.

Furthermore, there was an important relationship between the male gender and the relative skeletal muscle index (RSMI). In the female gender, no increase was observed. Statistical significance was evidenced by the combinations of supplements creatine, branched-chain amino acids (BCAA), Whey, and Whey, creatine, and beta-alanine. There was no statistically significant difference between the mean RSMI values of the DEXA and BIO groups.

Keywords: Supplements. Relative skeletal muscle index. Exercise. RMSI. Muscle mass.

Introduction

The use of dietary supplements is very common throughout the world, and Brazil is no different, being the third country in terms of dietary supplement users. Indiscriminate use is a problem for overall health, but when indicated and used correctly, it can influence muscle mass gain, performance, and overall health, being essential for some athletes to meet their needs. In 1998, the Ministry of Health, in order to avoid the indiscriminate consumption of dietary supplements, published Ordinance No. 222, defining guidelines for the legal use of dietary supplements among those who practice physical activity **[1,2]**.

In 2006, there was a change, with the publication of Ordinance No. 354, which establishes guidelines for the use of supplements for the athlete public **[3]**. When a patient seeks a supplement, they are also largely seeking to gain lean mass **[4-10]**. This study sought to demonstrate a relationship between the increase in lean mass as measured by the relative skeletal muscle mass index (RSMI) and the use of the most frequent supplements in the studied population. It also demonstrated, in numbers and in a more tangible way, the relationship between physical exercise and the use of supplements most commonly used in clinical practice, such as whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine, with the relative skeletal muscle indices in physically active adults according to the criteria of the World Health Organization (WHO) through the validated anthropometric method such as electrical bioimpedance or dual-energy X-ray absorptiometry (DEXA).

The relationship, if positive in the results, may demonstrate not only the importance of follow-up with a health professional in improving body composition by the RSMI in the correct use of supplementation, but also whether there is any relationship with the type of supplement used. If a relationship exists, which supplements are associated with a higher RSMI value? The project also seeks to assess the prevalence of supplement use in this population, as well as which supplement is most commonly used.

The overall objective was to evaluate whether there is a relationship between physical exercise and the use of exercise-related supplements most commonly used in clinical practice with the RSMI, while the specific objectives were to determine the prevalence of dietary supplement use and to evaluate the mean RSMI value in adults.

Methods

Study Design

This study followed a retrospective observational model, adhering to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. Available at: https://www.strobe-statement.org/checklists/. Accessed on 10/10/2024. The overall objective was to evaluate whether there is a relationship between physical exercise and the use of exercise-related supplements most commonly used in clinical practice (whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine) with relative skeletal muscle index (RSMI). The specific objectives, aligned with the overall objective, were mainly to determine the prevalence of dietary supplement use through anamnesis conducted in medical consultations for physically active patients according to World Health Organization (WHO) criteria, and to evaluate the average value of the relative skeletal muscle index in adults using validated anthropometric methods such as bioimpedance analysis or Dual Energy X-ray (DEXA) in patients who meet the same previous criteria.

Ethical Approval

This retrospective study was approved by the ethics committee of the European University of the Atlantic, Program for Mastering Nutrition, Physical and Sports Activities, C. Isabel Torres, 21, 39011 Santander, Cantabria, Spain, in accordance with the prerogatives of the Declaration of Helsinki, adopted in 1964 by the World Medical Association (WMA), and updated in October 2024 during the 75th General Assembly in Helsinki. The retrospective study was conducted without the requirement for an Informed Consent Form. This exemption is due to the fact that it is a retrospective study based on the evaluation of previously collected medical records that do not identify or expose the identity of patients, fully preserving the anonymity and image of the subject as well as their non-stigmatization, with the data obtained in the research being used only for the linked project.

Population and Sample

Fifty adult patients aged 18 to 65 years were selected from the Confiance medical clinics, located in the Vitrium Complex, and the Atrios Clinic, located in the same city in the Central Clínico Sul Complex, who exercised and used at least one dietary supplement, and who were seen between May 1, 2023, and May 1, 2024.

Patients under 18 or over 65 years of age, patients who did not engage in at least 150 minutes of moderate exercise per week (sedentary), and those who did not use at least one sports supplement, as well as those patients whose medical records did not contain accurate information on the use of the supplement(s), were excluded. The average annual patient population at the aforementioned clinics is 4,000. Of these, the chosen sample consisted of the first 50 adult patients who met the necessary criteria listed below, aged between 18 and 65 years, who practiced physical exercise and were evaluated at medical centers in the city of Brasília between May 2023 and May 2024 through Bioimpedance Analysis or whole-body DEXA. Only patients who used one or more supplements and were not sedentary according to the health organization's criteria were included in the study. Patients who did not meet these requirements were excluded.

Variables

The variables used were: Age (18 to 65 years), selecting the adult age range; Sex: Male or Female; Supplement used: Whey, Creatine, Beta-alanine, HMB, Glutamine, and Caffeine, being the most common reported by the studied population; and finally, the presented RSMI value. Table 1 demonstrates the operationalization of the variables and defines the concept, description, and indicators of the variables used.

Table 1. Operationalization of the variables.

Variable	Concept	Description	Indicators
Age (Quantitative)	Lifespan of an individual	Age expressed in years of the individual	Minimum age = 18 years. Maximum age = 65 years.
Sex (Qualitative)	Biological sex of the individual	Biological sex	Male or Female
Amount of activity performed per week (Quantitative)	Number of days per week that physical activities are practiced	Number of times of exercise practiced per week	3 to 9 times per week.
Supplement used (Qualitative)	Sports nutritional supplements used as a complement to the diet	Main sports supplements in clinical practice	Creatine, Beta-alanine, Whey Protein, Glutamine, HMB and Caffeine
Relative skeletal muscle index (RSMI) (Quantitative)	The relative skeletal muscle index calculation is the value obtained by dividing the lean mass of the lower and upper limbs (appendicular lean mass) by the height squared.	RSMI expressed in Kg/m^2	Normal value if ≥ 7.26 kg/m^2 for men and ≥ 5.45 kg/m^2 for women

Source: Own authorship.

Measurement Instruments and Techniques

The questionnaires used were medical anamnesis forms directed towards the medical specialties of Nutrition and Endocrinology, which included the type of exercise practiced, time and intensity, use of medications and dietary supplements (including dose, type, frequency), among other information not pertinent to the study. The World Health Organization's recommendation for physical activity was used as the criterion for sedentary lifestyle, and the evaluation was done through direct questions to the patient about their exercise practice. The tools used to measure anthropometric data were the Lunar Prodigy Primo whole-body DEXA (Figure 1) or the Inbody 370S bioimpedance (Figure 2).

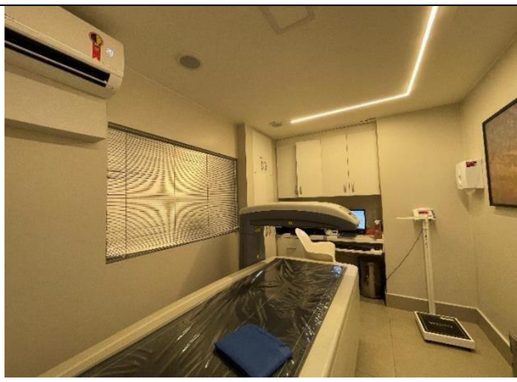

Figure 1. Full-body dexamethasone of the Lunar Prodigy Primo model. Source: Own authorship.

Figure 2. Bioimpedance model Inbody 370S. Source: Own authorship.

Procedures and Data Collection

The procedure adopted was an intervention with the study population carried out through the review of medical records of patients from two medical centers in the city of Brasília / Brazil. Data were collected during medical consultations, after selecting the records of patients who met the requirements for the study (age between 18 and 65 years, physically active, and who had undergone some validated anthropometric method such as DEXA or Bioimpedance Analysis). The data were organized according to the use or non-use of supplements, the amount of exercise practiced per week, and, if supplements are used, the type, frequency, and dose of supplement used were reported. The information will be organized in tables. SPSS may be used for statistical data or other programs.

This study was conducted using the waiver of the informed consent form presented at the end of this work, as it is a retrospective study based on the evaluation of previously collected medical records that do not identify or expose the identity of patients, fully preserving the anonymity and image of the subject, as well as their nonstigmatization. The data obtained in the research were only used for the linked project, and it was not possible to collect signatures from all patients already treated.

Statistical Analysis

The study followed a retrospective observational model, following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. Apple Pages software was used to organize and analyze the collected data, along with Apple Numbers and Microsoft Excel software, complemented by IBM SPSS Statistics statistical software. Other programs could also be used for tables and analyses. Various types of analyses were applied to the variables in order to find the desired data aligned with the general and specific objectives, validated by proven methods, such as calculating the average age of the participants, average RSMI by sex, average RSMI (relative skeletal muscle mass index) in the DEXA (whole body DEXA) and BIO (bioelectrical impedance analysis) groups, and also comparing the values found by One-Way ANOVA (one-way analysis of variance). The Pearson Chi-Square Test was also used in the data analysis, and nominal logistic regression analysis of the general supplement predictors (DEXA and BIO) in relation to the RSMI response predictor was performed. Through binary logistic regression analysis, the existence of a relationship between gender and RSMI was evaluated.

Results

Table 2 presents the general clinical data, with a total of 50 participants, the majority being male (58%), as well as the results obtained in the DEXA (n=44 participants) and BIO (n=06 participants) groups. The overall mean age was 40.60±8.95 years (22 to 60), being 40.72±8.32 years for men and 40.38±9.97 for women, with p=0.895>0.05, with no statistically significant difference. Table 2 also shows that the amount of physical exercise practiced per week was 4.80±1.43 (3 to 9) overall, being 4.89±1.39 for the male gender and 4.67 ± 1.49 for the female gender, with no statistically significant difference (p=0.855>0.05). It was also found that the overall relative skeletal muscle index (RSMI) had a mean of 8.56±1.76, with males at 9.68±1.10 and females at 7.03 ± 1.28, with a statistically significant difference (p=0.000).

In the DEXA group, the mean age was 40.30 ± 9.02, with the majority being male (60%). The overall mean number of exercises per week was 4.89 ± 1.45. The mean RSMI values were 9.45±1.16 for males and 7.01±1.21 for females, with a statistically significant difference (p=0.000).

For the BIO group, the mean age was 42.67±8.94, with males comprising 50%. The overall average of exercises per week was 4.17±1.17. The mean RSMI values were 9.29 ± 0.71 for males and 6.45±0.83 for females, with a statistically significant difference (p=0.000).

Table 2. General clinical data and p-value results according to the parametric One-Way ANOVA test, with p<0.05 showing a statistically significant difference at the 95% CI.

GENERAL CLINICAL DATA (n=50 participants)		**p-value**
Age (years)	Mean: 40.60 ± 8.95 years (22 to 60) Male: 40.72 ± 8.32 years (28 to 55) Female: 40.38 ± 9.97 (22 to 60)	0.895
Gender (M=male; F=Female)	General: M: 29 (58%); F: 21 (42%)	
Exercises per week	Mean: 4.80 ± 1.43 (3 to 9) Male: 4.89 ± 1.39 (3 to 8) Female: 4.67 ± 1.49 (3 to 9)	0.855
RSMI	Mean_general: 8.56 ± 1.76 (5.8 to 12.6) Male: 9.68 ± 1.10 (7.11 to 12.56) Female: 7.03 ± 1.28 (5.81 to 10.56)	0.000
Anthropometric assessment	DEXA: 44 (88%); Bio: 06 (12%)	
DEXA (n=44 participantes)		**p-value**
Age (years)	Mean: 40.30 ± 9.02 (22 to 60)	
Gender (M=Male; F=Female)	M: 26 (60%); F: 18 (40%)	
Exercises per week	Mean: 4.89 ± 1.45 (3 to 9)	
RSMI	Mean: 8.66 ± 1.76 (5.81 to 12.56) Male: 9.45 ± 1.16 (7.14 to 12.56) Female: 7.01 ± 1.21 (5.79 to 10.56)	0.000

BIO (n=06 participants)		p-value
Age (years)	Mean: 42.67 ± 8.94 (27 to 53)	
Gender (M=Male; F=Female)	M: 3 (50%); F: 3 (50%)	
Exercises per week	Mean: 4.17 ± 1.17 (3 to 6)	
RSMI	Mean_general: 7.87 ± 1.70 (5.93 to 9.77) Male: 9.29 ± 0.71 (8.47 to 9.77) Female: 6.45 ± 0.83 (5.93 to 7.41)	0.000

Note: RSMI: normal > 7.26 kg/m² in men and > 5.45 kg/m² in women. Source: Own authorship.

Figures 3 to 5 show the types and percentage (%) of dietary supplement use by participants. Figure 3 demonstrates the overall use of supplements (DEXA and BIO), showing that creatine (34%) and creatine with whey protein (24%) were the most frequently used, with a statistically significant difference compared to other supplement uses, according to Pearson's Chi-Square test, with p>0.05 (not rejecting the null hypothesis H_0). Figure 4 presents the results for the DEXA group, where creatine (36.4%) and creatine with whey protein (27.3%) were the most frequently used, with a statistically significant difference compared to other supplement uses, according to Pearson's Chi-Square test, with p>0.05 (not rejecting the null hypothesis H_0). Figure 5 represents the results for the BIO group, with no difference from the use of supplements (p<0.05, rejecting the null hypothesis H_0).

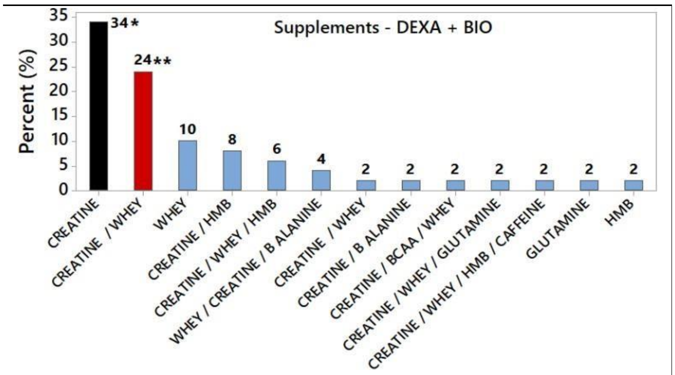

Figure 3. Overall results (DEXA and BIO) of supplement use. Source: Own authorship.

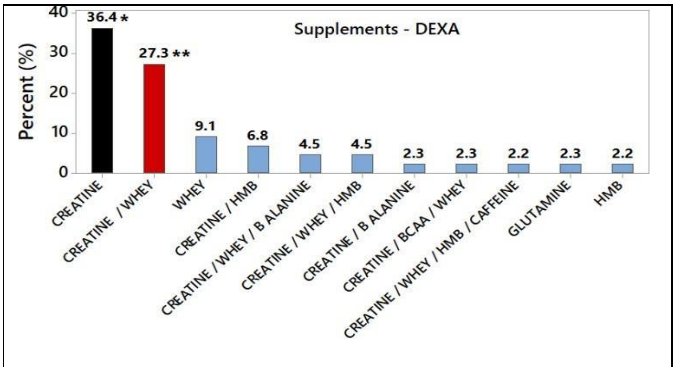

Figure 4. Results from the DEXA group using the supplements. Source: Own authorship.

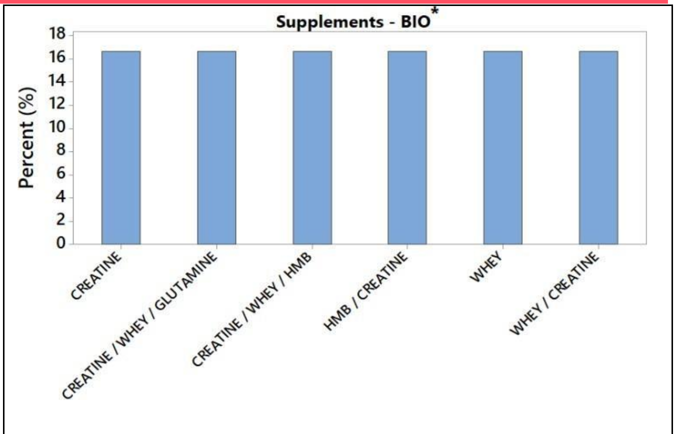

Figure 5. Results from the BIO group regarding the use of supplements. Source: Own authorship.

Note: * and **Pearson's Chi-Square test, with p<0.05 indicating a statistically significant association (rejecting the null hypothesis H_0).

Through binary logistic regression analysis, it was observed that there was an important relationship between the male gender and the relative skeletal muscle index (RSMI), with OR=4.89 and p=0.000 (Table 3).

Table 3. Binary logistic regression analysis of overall male and female genders (DEXA and BIO) in relation to RSMI, with p<0.05 significant.

Variables	*DF*	*Chi-Square*	*Odds Ratio (OR)*	*95% CI*	*p-value*
Male vs. RSMI	1	36,46	4,8904	(2,2071, 10,8361)	0,000
Female vs. RSMI	1	36,46	0,2045	(0,0923, 0,4531)	0,000

Source: Own authorship.

Figure 6 represents the predictive logistic regression analysis of the overall age (years) predictor (DEXA and BIO) in relation to the RSMI response predictor, without statistical significance (p=0.991).

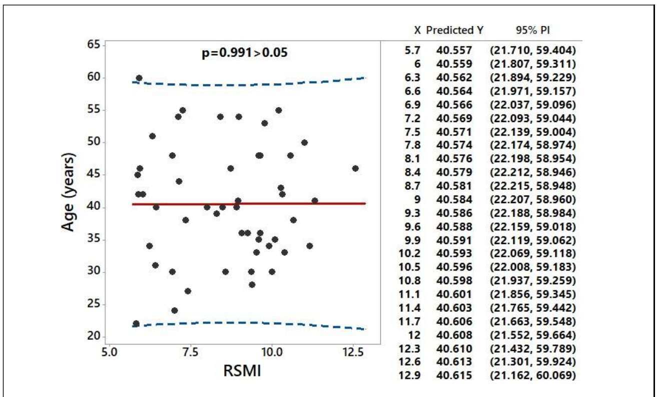

Figure 6. Result of the predictive logistic regression analysis of the overall age (years) predictor (DEXA and BIO) in relation to the RSMI response predictor, with p<0.05 significant. Source: Own authorship.

Figure 7 represents the predictive logistic regression analysis of the overall exercise/week predictor (DEXA and BIO) in relation to the RSMI response predictor, with no statistical significance (p=0.237).

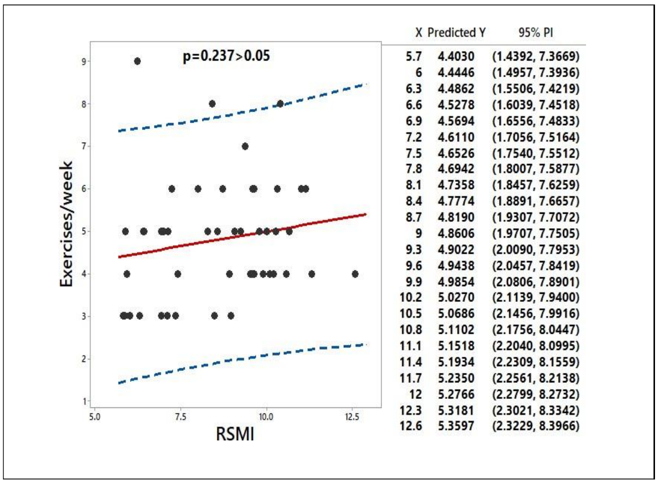

Figure 7. Result of the predictive logistic regression analysis of the overall exercise/week predictor (DEXA and BIO) in relation to the RSMI response predictor, with p<0.05 significant. Source: Own authorship.

Table 4 presents the nominal logistic regression analysis of the general supplement predictors (DEXA and BIO) in relation to the RSMI response predictor, with statistical significance in relation to the combination of creatine, branched-chain amino acids (BCAA), whey, and whey, creatine and beta-alanine supplements, showing p=0.015 and OR=1.62 (in red) for both combinations of supplements used by the participants.

Table 4. Results of the nominal logistic regression analysis of the general supplement predictors (DEXA and BIO) in relation to the RSMI response predictor, with p<0.05 significant.

Predictors	Z	P	Odds Ratio (OR)
Logit 1: (WHEY/WHEY / CREATINE / B ALANINE)			
RSMI	-0.08	0.934	0.96
Logit 2: (HMB/WHEY / CREATINE / B ALANINE)			
RSMI	-1.12	0.263	0.17
Logit 3: (GLUTAMINE/WHEY / CREATINE / B ALANINE)			
RSMI	-1.12	0.261	0.34
Logit 4: (CREATINE / WHEY / HMB / CAFFEINE/WHEY / CREATINE / B ALANINE)			
RSMI	-1.15	0.249	0.20
Logit 5: (CREATINE / WHEY / HMB/WHEY / CREATINE / B ALANINE)			
RSMI	-0.66	0.512	0.68
Logit 6: (CREATINE / WHEY / GLUTAMINE/WHEY / CREATINE / B ALANINE)			
RSMI	-0.96	0.335	0.45
Logit 7: (CREATINE / HMB/WHEY / CREATINE / B ALANINE)			
RSMI	-1.45	0.146	0.42
Logit 8: (CREATINE / BCAA / WHEY and WHEY / CREATINE / B ALANINE)			
RSMI	0.53	0.015	1.62
Logit 9: (CREATINE / B ALANINE/WHEY / CREATINE / B ALANINE)			
RSMI	-0.04	0.972	0.97
Logit 10: (CREATINE / WHEY/WHEY / CREATINE / B ALANINE)			
RSMI	0.10	0.919	1.09
Logit 11: (CREATINE / WHEY/WHEY / CREATINE / B ALANINE)			
RSMI	-0.83	0.405	0.65
Logit 12: (CREATINE/WHEY / CREATINE / B ALANINE)			
RSMI	-0.44	0.659	0.80

Source: Own authorship.

Figure 8 shows the comparative results between the mean RSMI values for the DEXA (8.66) and BIO (7.87) groups using the One-Way ANOVA test. No statistically significant difference was observed between the mean RSMI values of the DEXA and BIO groups, with p=0.307>0.05 and a difference between the mean values of -0.789.

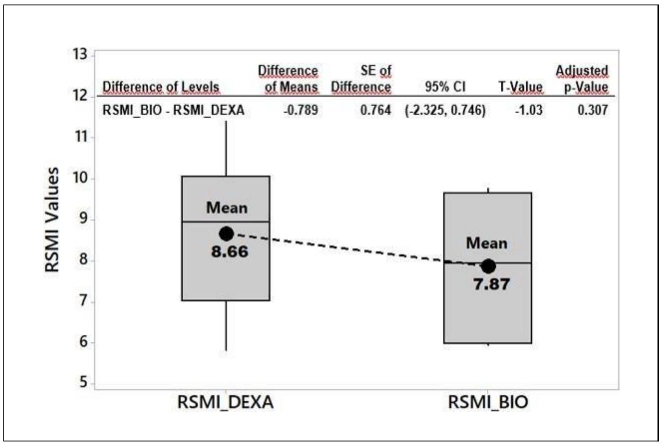

Figure 8. Box-plot graph showing the comparative results of the mean RSMI values for the DEXA and BIO groups. A p-value <0.05 was considered significant by the One-Way ANOVA test. Source: Own authorship.

Discussion

This study demonstrated the relationship between physical exercise and the use of supplements most commonly used in clinical practice, such as whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine, with the relative skeletal muscle mass index (RSMI) in physically active adults according to the World Health Organization (WHO) criteria. The validated anthropometric methods employed were bioelectrical impedance and dual-energy X-ray absorptiometry (DEXA).

As an outcome, the general clinical data showed a total of 50 participants, the majority being male (58%), with the DEXA group having 44 participants and the BIO group having 6 participants. The overall average age was 40.60 years (22 to 60). The number of physical exercises performed per week was 4.80 (3 to 9) in general. It was also found that the overall RSMI had an average of 8.56 ± 1.76. The overall use of supplements (DEXA and BIO) showed that the use of creatine (34%) and creatine with whey protein (24%) were the most frequent.

Also, through binary logistic regression analysis, the present study observed that there was an important relationship between the male gender and the relative skeletal muscle index (RSMI), with OR=4.89 and p=0.000, that is, the male gender had about five times greater chances of having an increased RSMI. No increase was observed in the female gender.

The nominal logistic regression analysis of the general supplement predictors (DEXA and BIO) in relation to the RSMI response predictor showed statistical significance in relation to the combinations of creatine, BCAA, Whey, and Whey, creatine and beta-alanine supplements, presenting p=0.015 and OR=1.62 for both combinations, that is, the combination of these supplements showed 1.62 times greater chances of impacting the increase in RSMI.

This study also showed, through the comparative result between the mean RSMI values in relation to the DEXA (8.66) and BIO (7.87) groups, using the One-Way ANOVA test, that there was no statistically significant difference between the mean RSMI values of the DEXA and BIO groups, with p=0.307. In addition to these results from the present study, several studies have been published showing the positive relationship between dietary supplements and RSMI **[11-14]**.

As an important example, a longitudinal study analyzed the relationship between genes related to muscle performance and the risk of increased BMI and muscle mass and decreased fat mass in professional soccer players after creatine supplementation. A total of 161 male professional soccer players were recruited. The creatine supplementation protocol consisted of 20 g/day of creatine monohydrate for 5 days (loading dose) and 3-5 g/day for 7 weeks (maintenance dose). Anthropometric characteristics BMI, fat mass, and muscle mass were recorded before and after the creatine supplementation protocol. The results showed that the allelic frequencies of ACE and AMPD1 differed between responders and non-responders in the increase in muscle mass (all $p<0.05$). Players with a Tekken God Supreme (TGS) greater than 54.16 a.u. had an odds ratio (OR) of 2.985 (95% CI: 1.560-5.711; p=0.001) for increased muscle mass. In contrast, those with a TGS below 54.16 a.u. had an OR of 9.385 (95% CI: 4.535-19.425; $p < 0.001$) for suffering non-contact muscle injuries during the season. Thus, the increase in BMI and muscle mass in response to creatine supplementation in professional soccer players was influenced by a TGS derived from the combination of favorable genotypes linked to muscle performance **[15]**.

Also, advancing age is associated with lower levels of physical activity, suboptimal protein intake, and desensitization to anabolic stimuli, predisposing to age-related muscle loss (sarcopenia). Although resistance exercise (RE) and protein supplementation partially protect against sarcopenia under controlled conditions, the efficacy of unsupervised home RE (HBRE) and multi-ingredient supplementation (MIS) is largely unknown. A randomized, placebo-controlled, double-blind trial analyzed the effects of HBRE/MIS on muscle mass, strength, and function in active older men. A total of 32 sedentary men underwent twelve weeks of home resistance band training (3 days/week) in combination with daily intake of a novel five-nutrient supplement (Muscle5; M5, n = 16, 77.4 ± 2.8 years) containing whey, micellar casein, creatine, vitamin D, and omega-3 fatty acids, or an isocaloric/isonitrogenic placebo (PLA; n = 16, 74.4 ± 1.3 years) containing collagen and sunflower oil. Appendicular and total lean mass (ASM; +3%, TLM; +2%), lean mass to fat ratios (ASM/% body fat; +6%, TLM/% body fat; +5%), maximum strength (grip; +8%, leg press; +17%) and function (5-time to sitto-stand; -9%) were significantly improved in the M5 group after HBRE/MIS therapy (pre vs. post-tests; $p < 0.05$). Cross-sectional areas of fast-twitch muscle fibers in the quadriceps muscle were also significantly increased in the M5 group after the intervention (Type IIa; +30.9%, Type IIx, +28.5%, $p < 0.05$). Subgroup analysis indicated even greater gains in total lean mass in sarcopenic individuals after HBRE/MIS therapy (TLM; +1.65 kg/+3.4%, $p < 0.05$) **[16]**.

Similarly, the present study also observed a significant increase in RSMI (with OR=4.89 and p=0.000) in men of all ages. The authors O'Bryan et al. (2021) **[17]** through a meta-analysis study, analyzed the effects of multi-ingredient protein (MIP) supplements on muscle mass and strength gains induced by resistance exercise training (RT) compared to protein-only supplementation (PRO) or placebo. The most common MIP supplements included protein with creatine (n=17) or vitamin D (n=10). Data from 35 trials with 1387 participants showed significant increases ($p<0.05$) in lean mass (0.80 kg (95% CI 0.44 to 1.15)), lower body muscle mass (4.22 kg (95% CI 0.79 to 7.64)) and upper body muscle mass (2.56 kg (95% CI 0.79 to 4.33)) where a supplement was compared with all nonsupplemented MIP conditions (means (95% CI)). Subgroup analyses indicated a greater effect of MIP supplements compared with all non-MIP supplements on lean mass in untrained participants (0.95 kg (95% CI 0.51 to 1.39), $p<0.0001$) and older participants (0.77 kg (95% CI 0.11 to 1.43), p=0.02); Taking MIP supplements was also associated with gains in upper body muscle mass (1.56 kg (95% CI 0.80 to 2.33), p=0.01) in older adults. When MIP supplements were combined with resistance exercise training, there were greater gains in lean mass and strength in healthy adults than in counterparts who were supplemented with non-MIP. MIP supplements were not superior when directly compared with PRO supplements.

A meta-analysis study conducted by the authors Li et al. (2024) **[18]** analyzed the effects of Whey Protein (WP) enriched supplement intake with or without resistance training (RT) in elderly patients. A total of 10 RCT studies, including 1154 participants, were included and analyzed. The primary outcomes were changes in muscle mass, strength, and physical performance. In the WP group versus (vs.) isocaloric placebo (PLA)/routine visit (RC), WP significantly increased appendicular skeletal muscle mass index, appendicular skeletal muscle mass, and gait speed in older patients with sarcopenia. In the WP group with RT vs. the PLA/RC group, there was a significant increase in handgrip strength. Furthermore, in secondary outcomes, WP significantly reduced interleukin-6, significantly increased insulin-like growth factor-1 and albumin, promoted participants' total energy and protein intake, improved activity of daily living scores in patients, and had no significant effect on BMI, weight, or fat mass.

A randomized, placebo-controlled clinical trial conducted by authors Bo Yet et al. (2019) **[19]** evaluated the effect of a nutritional supplement containing Whey Protein, vitamin D, and E on measures of sarcopenia. A total of 60 older adult sarcopenic individuals participated in the current randomized, double-blind, placebo-controlled (isocaloric control product) study for 6 months. RSMI measured by bioimpedance analysis (BIA)), muscle strength (handgrip strength), physical function (6-m gait speed, chair stand test, and timed stand-up walk test, TUG), quality of life (measured by the Short-Form 36-Item Health Survey, SF-36), and blood biochemical indices were measured before and after the 6-month intervention. Compared to the placebo group, nutritional supplementation improved RSMI (mean difference: 0.18 kg/m2, 95% CI: 0.01-0.35, p=0.040), handgrip strength (mean difference: 2.68 kg, 95% CI: 0.71-4.65, p=0.009), and the SF-36 mental component summary (SF-36 MCS) (difference Mean difference: 11.26, 95% CI: 3.86-18.65, p=0.004), the summary of the physical component of the SF-36 (SF-36 PCS) (mean difference: 20.21, 95% CI: 11.30-29.12, p<0.001), serum IGF-1 (mean difference: 14.34 ng/mL, 95% CI: 2.06-26.73), IL-2 (mean difference: -575.32 pg/mL, 95% CI: -1116.94 ~ -33.70, p=0.038), serum vitamin D3 (mean difference: 11.01 ng/mL, 95% CI: 6.44-15.58, p<0.001) and serum vitamin E (mean difference: 4.17 ng/L, 95% CI: 1.89-6.45, p=0.001). Thus, this study demonstrated that combined supplementation with whey protein, vitamin D, and vitamin E can significantly improve RSMI, muscle strength, and anabolic markers such as IGF-I and IL-2 in older adults with sarcopenia. These results are also congruent with the results of this study, since in addition to the present study showing an almost five-fold increase in RSMI with the use of supplements in men, it also showed an important result with the combinations creatine, BCAA, Whey, and Whey, creatine, and beta-alanine, presenting p=0.015 and OR=1.62 for both.

In addition, another randomized, placebo-controlled clinical trial tested the hypothesis that nutritional supplementation with Whey Protein (22g), essential amino acids (10.9g, including 4g of leucine) and vitamin D [2.5µg (100 IU)] concomitantly with regular and controlled physical activity would increase fat-free mass, strength, physical function, and quality of life, and reduce the risk of malnutrition in sarcopenic elderly individuals. A total of 130 sarcopenic elderly individuals (53 men and 77 women; mean age: 80.3 years) participated in the study for 12 weeks. All participants simultaneously participated in a controlled physical activity program. Body composition was examined using dual-energy X-ray absorptiometry, muscle strength with a handgrip dynamometer, and blood biochemical indices of nutritional and health status were assessed, and overall nutritional status, physical function, and quality of life were evaluated before and after the 12-week intervention. Compared with physical activity and placebo, supplementation plus physical activity increased fat-free mass (gain of 1.7 kg, p<0.001), muscle mass relative skeletal muscle (p=0.009), android fat distribution (p=0.021), handgrip strength (p=0.001), standardized summary scores for physical components (p=0.030), activities of daily living (p=0.001), mini nutritional assessment (p=0.003) and insulin-like growth factor I (p=0.002) and reduced C-reactive protein (p=0.038) **[20]**.

A randomized, double-blind clinical trial conducted by the authors Colonetti et al. (2023) **[21]** examined the effect of whey protein and vitamin D supplementation on body composition and skeletal muscle in older adults. A total of 30 older adults (>60 years) were randomized and allocated into three groups: a group receiving resistance training and supplementation; a group receiving resistance training, whey protein, and vitamin D; a group receiving resistance training and a placebo; and a control group with no intervention. Body composition was measured by dual-energy X-ray absorptiometry at baseline, 12 weeks, and 24 weeks. The mean age was 74.87 (± 8.14) years. A significant difference (p=0.042) was observed between the group receiving resistance training and supplementation and the control groups regarding the increase in lean mass (kg) at 24 weeks. After 24 weeks of intervention, there was a significant increase in the relative muscle mass index for both groups that performed resistance training, the group that received resistance training and placebo (p=0.042), and the group that received resistance training and supplementation (p=0.045) compared to the control group. The present study showed that the combination of creatine, BCAA, Whey, Whey/creatine, and beta-alanine supplements presented the best results in increasing RSMI (OR=1.62).

As proof of this, the aforementioned studies showed that creatine and Whey Protein supplements can increase RSMI in patients of various age groups and genders, especially in the elderly. And other studies have also shown the same effects in relation to other supplements such as BCAA and beta-alanine, both in isolation and in combination with other nutrients and vitamins. As a corollary to this, a randomized, placebo-controlled clinical trial evaluated the acute effects of a single carnitine and beta-alanine (Carn-βA) supplementation on the cardiorespiratory and metabolic response during a ramp cycle ergometry test. A total of 10 healthy men were included (age: 22.2±1.9 years, body mass: 72.5±7.9 kg, Body Mass Index: 24.47±1.91 kg/m^2). All participants performed two maximal incremental ramp tests on a cycle ergometer, with a pre-randomized assumption of 2.5 g of L-carnosine plus 2.5 g of β-alanine (Carn-βA) or placebo (PLA). Although acute Carn-ßA supplementation did not affect individual ventilatory thresholds, the compensated portion of the ramp test (i.e., the difference between VT2 and VT1) was significantly higher (p=0.043) in Carn-ßA. These findings demonstrate a positive effect of acute Carn-ßA supplementation on the compensated portion of exercise **[22]**.

Besides, chronic β-alanine (BA) supplementation is an increasingly popular nutritional strategy because it can raise muscle carnosine content and thus improve performance in high-intensity exercise. One study investigated whether gender and body mass are determinants of BA-induced muscle carnosine load and whether the ideal maintenance dose to ensure consistently high muscle carnosine stores is determined. A total of 34 participants (men and women) were supplemented with 3.2 g (4 × 800 mg) of BA per day for 46 days (slightly different loading strategies were applied regarding the effect of meal timing and form of supplementation). After that, 19 participants (men and women) continued taking free powdered BA for a further six weeks (maintenance phase). Participants were matched and redistributed into three groups that received 0.4, 0.8, and 1.2 g·d(-1) of BA, respectively. Muscle carnosine content was measured in the soleus and gastrocnemius muscles using proton magnetic resonance spectroscopy. Body mass and gender had only a minimal effect on the absolute increase in muscle carnosine. Given the lower baseline values in women, the relative increase for women was greater, indicating that women required less BA for the same relative increase. Furthermore, a significant negative correlation was observed between body mass and the relative increase in muscle carnosine (r = -0.45, p = 0.007). A maintenance dose of ~1.2 g·d(-1) BA was the most effective in maintaining elevated muscle carnosine content at the post-supplementation level. An effective maintenance dose of ~1.2 g·d(-1) BA to maintain muscle carnosine content elevated at 30%-50% above baseline for a prolonged period **[23]**.

A study determined whether an 8-hour infusion of BCAAs increases mitochondrial ATP production rate (MAPR) equally in healthy young and older adults. Using a crossover study design, the effect of BCAAs versus saline infusion was compared in 12 young (23.0 +/- 0.8 years) and 12 older (70.7 +/- 1.1 years) participants matched for sex and body mass index. Skeletal muscle MAPR and mitochondrial DNA (mtDNA) abundance were measured in muscle biopsy samples obtained before and at the end of the 8-hour infusion. In young participants, MAPR with the substrate glutamate plus malate (providing electrons to complex I) and succinate plus rotenone (complex II) increased in response to BCAA infusion, compared to a decline in MAPR in response to saline infusion. In contrast, MAPR was not affected by BCAA infusion in elderly participants. Furthermore, mtDNA abundance was lower in the elderly compared to young participants, but was not affected by BCAA infusion. BCAA does not increase muscle mitochondrial function in the elderly **[24]**.

The authors Yang et al. (2023) **[25]** evaluated through a randomized placebo-controlled clinical trial the impact of beta-hydroxy-beta-methylbutyrate (HMB) intervention on muscle strength, physical performance, body composition, and inflammatory factors in older adults with sarcopenia. Participants aged ≥60 years with sarcopenia were included and assigned to the HMB group (HMBG, n=18) and the placebo group (PG, n=16). Intervention: The HMBG and PG received HMB and placebo products twice daily for 12 weeks, and both received resistance exercise training twice weekly for 12 weeks. After the 12-week intervention, HMBG demonstrated significantly greater improvements in handgrip strength (4.61 (95% CI: 2.93, 6.28) kg, p<0.001), gait speed (0.11 (95% CI: 0.02, 0.20) m/s, p=0.014), five-times chair stand test (-3.65 (95% CI: -5.72, -1.58) s, p=0.001), muscle quality (2.47 (95% CI: 1.15, 3.80) kg.kg-1 p=0.001) and weak tumor necrosis factor-like apoptosis inducer (-15.23 (95% CI: -29.80, -0.66) pmol/mL, p=0.041) compared with PG; No significant differences in skeletal muscle mass, skeletal muscle index, and other body composition parameters were found between the two groups. HMB significantly enhances the effect of resistance exercise training on muscle strength, physical performance, muscle quality, and the reduction of inflammatory factors.

It has also been observed that the free acid β-hydroxy-β-methylbutyrate (HMB-FA) has been suggested to accelerate the regenerative capacity of skeletal muscle after high-intensity exercise and attenuate markers of skeletal muscle damage. In conjunction with resistance training, HMB-FA supplementation may attenuate markers of muscle damage, increase acute immune and endocrine responses, and improve training-induced muscle mass and strength. HMB-FA supplementation may also improve markers of aerobic fitness when combined with high-intensity interval training **[26]**.

One study examined the effect of caffeine supplementation on injured and uninjured muscles. Eight men and women (n=16) who were physically active individuals participated in this study (age: 24.3±4.3 years; height: 173.0±7.0 cm; mass: 75.2±11.5 kg; body fat: 18.2±15.9%). One leg was evaluated in uninjured and injured conditions (100 eccentric quadriceps contractions) after caffeine supplementation (6 mg·kg), with the other leg evaluated in both conditions after placebo supplementation. Compared with placebo, caffeine increased peak isokinetic torque by 6.8±2.3 and 9.4±2.5% in uninjured and injured muscles, respectively, but had no effect on maximum voluntary isometric torque or fatigue index in uninjured or injured muscles, with treatments exhibiting similar changes (p>0.05) in isometric torque (-11.9±2.2%), fatigue index (-13.9±3.4%) and pain (+44.0±4.7) after eccentric contractions. The results suggest that caffeine has a similar ergogenic effect on isokinetic torque in uninjured and injured states, but no effect on isometric torque production, pain perception, or degree of relative fatigue **[27]**.

In addition, glutamine is a non-essential amino acid predominantly biosynthesized in skeletal muscles, with a smaller fraction originating from organs such as the lungs, brain, liver, and adipose tissue **[28]**. During glutamine biosynthesis within skeletal muscle, BCAAs undergo deamination, transferring amino groups to α-ketoglutaric acid to produce glutamic acid. Subsequently, glutamic acid combines with ammonia to form glutamine through the catalytic activity of glutamine synthetase. Glutamine is one of the most abundant amino acids found in human tissues and organs, comprising 80% of free amino acids in skeletal muscles and 20% in plasma. Furthermore, it is a precursor for the biosynthesis of proteins, amino acids, nucleotides, and glucose. Research indicates that approximately 30–35% of the nitrogen resulting from protein degradation exists in the form of glutamine, highlighting its fundamental role in protein structure **[29]**.

Blood and skeletal muscle glutamine levels decrease rapidly during different forms of physical activity (e.g., resistance training and exhaustive exercise). These findings highlight the potential inadequacy of endogenous glutamine synthesis to meet the needs of athletes and individuals with specific medical conditions. Glutamine plays a role in immune system function, gut health, and overall protein balance **[30-32]**.

Finally, maintaining skeletal muscle mass is important for improving muscle strength and function. Therefore, maximizing lean body mass is the primary goal for both elite athletes and active or sarcopenic older adults. The use of amino acids as dietary supplements is widespread among athletes and physically active individuals. An extensive literature review reveals that BCAA, creatine, glutamine, and β-alanine may be useful in regulating skeletal muscle metabolism, improving lean body mass, and mitigating exercise-induced muscle damage **[33]**.

Limitations

Despite these findings, the present study had some limitations due to being a retrospective study with only 50 participants. Future randomized controlled studies are needed regarding the combinations of creatine, BCAA, whey, and whey, creatine, and beta-alanine supplements, as these showed important results in increasing the relative skeletal muscle mass index (RSMI).

Conclusion

It was concluded that there was a relationship between physical exercise and the use of supplements most commonly used in clinical practice, such as whey protein, creatine, hydroxymethylbutyrate, beta-alanine, glutamine, and caffeine, with the increase in the relative skeletal muscle mass index (RSMI) in physically active adults. The overall use of supplements (DEXA and BIO) showed that the use of creatine (34%) and creatine with whey protein (24%) were the most frequent. Furthermore, there was a significant relationship between the male gender and the relative RSMI. No increase was observed in the female gender. Statistical significance was found in relation to the combinations of creatine, BCAA, whey, and whey, creatine, and beta-alanine supplements. There was no statistically significant difference between the mean RSMI values of the DEXA and BIO groups.

CRediT

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Ethical Approval

This retrospective study was approved by the ethics committee of the European University of the Atlantic, Program for Mastering Nutrition, Physical and Sports Activities, C. Isabel Torres, 21, 39011 Santander, Cantabria, Spain, in accordance with the prerogatives of the Declaration of Helsinki, adopted in 1964 by the World Medical Association (WMA), and updated in October 2024 during the 75th General Assembly in Helsinki. The retrospective study was conducted without the requirement for an Informed Consent Form. This exemption is due to the fact that it is a retrospective study based on the evaluation of previously collected medical records that do not identify or expose the identity of patients, fully preserving the anonymity and image of the subject as well as their non-stigmatization, with the data obtained in the research being used only for the linked project.

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All referenced sources are accessible through the respective journals or public repositories.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

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Application of Artificial Intelligence (AI)

Not applicable.

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It was performed.

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